Supplementary Figure 1

A (i) 

B (i) 

(ii) 

Fluc/Rluc

0 0.02 0.04 0.06 0.08

pRp53(+39)F pRp53(-1)F pR-BiP-F pR-HAV-F

0 0.02 0.04 0.06 0.08

pRp53(-1)F pRp53(+39)F

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(ii) 

Fluc/Rluc

0 0.02 0.04 0.06 0.08

pRp53(-1)F pRp53(+39)F pR(+39)F (+1 ATGmut)

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Supplementary Figure 1. p53 IRESs show independent activity and similar efficiency to known viral and cellular IRESs (A i) Schematic representation of bicistronic plasmids used in transient transfections (ii) Luciferase assay after transfection of bicistronic plasmids containing p53 5′UTR (+39) or (-1), BiP IRES and HAV IRESs into HeLa cells. The Fluc/Rluc ratio is shown and the mean absolute values of Fluc and Rluc activity from each construct is depicted separately for each construct. (B i) Schematic representation of bicistronic plasmids used in transient transfections. The ATG at position +1 within the p53 +39 leader sequence was mutated to AAG in the pR(+39)F(+1ATGmut) construct (ii) Luciferase assay after transfection of the described bicistronic plasmids into HeLa cells. The data is depicted as in panel A. The data in both panels represent the mean ± SD of three independent experiments.
Supplementary Figure 2

A

Control

0 h

2 h

4 h

6 h

B

pRp53(+39)F

C

pRp53(-1)F

Relative Luc activity

Control

h after release

Control

h after release

Fluc

Rluc

Fluc

Rluc

h after release

Bar graphs showing relative luciferase activity for pRp53(+39)F (B) and pRp53(-1)F (C) constructs after different time points post-release.
Supplementary figure 2. Cell cycle-dependent p53 IRES activity in cells synchronized at G1/S phase. (A) Flow-cytometric analysis of HeLa cells collected at different time-points after being synchronized at G1/S phase by double thymidine treatment. (B) and (C) Luciferase assay of cells transfected with p53 (+39) and (-1) bicistronic constructs and synchronized at G1/S phase, collected at various time points after release. Fluc and Rluc activities at each time-point are expressed as fold of the Fluc and Rluc activity obtained from non-synchronized, transfected cells. The data represent the mean (± SD) of three independent experiments.
Supplementary Figure 3. Schematic representation of proposed cell cycle phase-dependent regulation of p53 IRES activity. The activity of the (+39) IRES, mediating the translation of ΔN-p53 is highest at the G1/S transition. The high level of ΔN-p53 generated at this stage negatively regulates the activity of full length p53, produced by cap-dependent translation. At the G2/M transition, cap-dependent translation of p53 is downregulated and full length p53 is translated via the (-1) IRES. Low activity of the (+39) IRES at this stage results in a low amount of ΔN-p53, which will not counteract the activity of full length p53 and allows the negative autoregulation of p53 mRNA translation by p53 protein.